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Current View

Heat Shock Proteins in Inflammatory Bowel Disease

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SUMMARY

Stress or heatshock proteins (HSPs) belong to five families of highly conserved intracellular proteins functioning as constitutive chaperones maintaining protein trafficking, structural integrity and repair. Their synthesis is induced by numerous stimuli ranging from environmental stress to inflammation regardless of etiology or malignancy. In normal mucosa HSP27 and HSP70 an normally expressed in the surface epithelium of the colon, an area continually subjected to inducible signals from the enteric flora and short chain fatty acids, rendering the epithelium resistant to bacterial toxins and inflammation associated stress. In inflammatory bowel disease (IBD) the heatshock proteins may play in important role as there is accumulating evidence that they suppress proinflammatory genes relevant to its pathogenesis. The mechanisms of suppression or induction of HSPS in inflamed gut mucosa need further research with the ultimate purpose that these molecules could be a target for therapeutic interventions.

Key Words: Heat Shock Proteins, Inflammatory Bowel Disease, Crohn's, Ulcerative Colitis

Stress or heat shock proteins (HSPs) are molecular chaperones that were discovered in 1962 as a set of highly conserved intracellular proteins, present constitutively in most mammalian and also prokaryotic cells [1,2]. Mammalian HSPs have been classified into five families according to their molecular size: HSP100, HSP90, HSP70, HSP60 and the small HSPs (15 to 30 kDa, including the HSP27). High molecular weight HSPs are ATP dependent chaperones, while small HSPs act in an ATP independent fashion. These chaperones are instrumental for signalling and protein traffic, even in the absence of stress. Their house keeping functions include trafficking of proteins between intracellular compartments, folding of proteins and refolding of misfolded proteins as well as degradation of protein complexes. Heat shock protein 10 (Hsp10) forms a complex with Hsp60 that is believed to be responsible for accelerating the folding of polypeptides imported into mitochondria, as well as reactivation of denaturated proteins, and diminishing aggregation of non-native polypeptides and partially unfolded kinetically trapped intermediates. The same complex has been shown to be up-regulated in pre-cancerous and cancerous cells.

Nevertheless the need for HSPs increases after proteotoxic damage. Their synthesis is induced by a variety of stimuli including thermal stress, heavy metals such as sodium arsenite and zinc ions, bacteria, and bacterial exo- and endotoxins, viral infections, ischemia, nutritional deficiency, ionizing radiation, oxidants, some IFN inducers, and cytokines[3]. Their activation occurs after transactivation of the genes by a family of DNA-binding proteins called the Heat Shock Factors (HSFs) (HSF1–4, of which the best known is HSF1). In unstressed cells the inactive HSF is bound to the cytoplasmic HSP40 (Hdj-1), HSP70, and HSP90 in a monomeric form without the DNA-binding activity. In response to stress, HSF is released and translocated into the nucleus, where it assembles into a trimer and binds to a specific consensus heat shock regulatory element (HSE) in the heat shock gene promoter to exert the transcriptional activation.[4,5]

The most highly inducible heat shock proteins belong to the HSP70 family, which includes both constitutive proteins (HSP70c) essential for cellular function and the

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inducible form (HSP70i), which increases in response to environmental stress. Among the five families of HSPs, HSP27 and HSP70 have been shown to have a strong association with cancer.

Recently it was discovered that the HSP70i and HSP70c are heat shock proteins encoded by highly related but differently regulated genes of the HSP70 multigene family. The HSP70i genes are expressed at a very low level at physiological temperature and are highly induced by heat shock and by many molecular stressors. In contrast, the HSP70c genes are constitutively expressed at physiological temperature and their activity remains unaffected by heat shock. It has been also suggested that HSP70c and HSP70i can be expressed in a regional and cell type–specific manner at physiological temperature in vivo. Especially important seems to be the observation that stress-inducible HSP70i, but not the constitutive HSP70c, co-segregates with immunogenicity of tumor cells in vivo and that the association with peptides is more pronounced for HSP70i than for HSP70 in various conditions.

Moreover heat shock proteins are thought to participate directly in antigen presentation of damaged proteins and may act as autoantigens.[6] Heat shock proteins (HSP) produced by bacterial agents may for instance induce an autoimmune response. Autoantibodies and reactive T-cells against different heat shock proteins have been found in various diseases including inflammatory bowel disease and may be caused by molecular mimicry due to the high grade homology between human and microbial, especially mycobacterial HSPs.

Mucosal injury and inflammation are cardinal manifestations of inflammatory bowel diseases (IBD), arising when the cell's capacity for defence (i.e. cytoprotection) or repair is overwhelmed. Inflammatory bowel disease (IBD) is a typical longstanding inflammatory disease of the gut with increased risk for the development of colorectal carcinoma and its two principal forms are Crohn's disease (CD) and ulcerative colitis (UC). In chronic inflammatory bowel diseases healing does not occur or is incomplete. The major question therefore in Crohn's disease and ulcerative colitis is why the early lesions do not heal but lead to chronic inflammation. Intrinsic defects, such as epithelial cell alterations, increased permeability or genetic predisposition are considered as possible causes. Persistent inflammation may also be due to the nature of an extrinsic agent or pathogen. A specific agent has as yet not been demonstrated in chronic idiopathic inflammatory bowel disease but inappropriate inflammatory reaction to components normally present in the lumen of the gastrointestinal tract must also be considered.

In normal intestinal mucosa, HSP27 and HSP70 are expressed "constitutively" in the surface epithelium of the colon, areas that are continuously subjected to inducing signals from enteric flora and short chain fatty acids. Maintained expression of HSP27 and HSP70 contributes to colonic epithelial resistance to bacterial toxins and inflammation-associated stress. In the context of chronic inflammation associated with IBD, the down-regulation of critical cytoprotective proteins, such as induced HSP (iHSP), through translational inhibition could have negative consequences. Their compromised expression renders the mucosa highly susceptible to inflammatory and immune processes and potentates otherwise harmless enteric flora[7]. Due to their pivotal role in maintaining intestinal homeostasis, the finding of reduced surface HSP27 and HSP70 expression in areas of mucosal inflammation in human IBD and experimental colitis were unanticipated,[7] however other reports had suggested increased expression in the areas of mucosa of HSP70 in UC, but not in CD, regardless of active disease.[8]

HSP32 is constitutively expressed in lamina propria inflammatory cells of normal gastric and colonic mucosa and in gastric epithelial cells. Upregulation of HSP32 expression occurred in inflamed gastric mucosa, whether or not infected by H pylori, and in the inflamed colon, particularly in patients with UC.[9] Because HSP32 is induced by oxidative stress, these results support the hypothesis that increased mucosal generation of reactive oxygen metabolites and other inflammatory mediators in inflamed gut mucosa could induce local synthesis of HSP32.[10]

The various HSP families are differentially expressed in the intestinal epithelial cells (IEC) and may relate to the type or intensity of the epithelial damage, or location of the IEC.[11] HSP60, HSP72, and HSP90 are expressed in the colonic mucosa after hyperthermia. In response to acetic acid–induced intestinal lesions, HSP72 and HSP90 are protective and their inductions precede that of HSP60, which by itself has no protective effect. In cases of functional diarrhea without histopathological changes HSP60 is induced but not HSP72 or HSP90. On the other hand, HSP72 and HSP73 have also been reported to have in both colon and small IEC no protective function against indomethacin-induced injuries[12]. The chaperone function for a particular HSP may, therefore, be specific to certain intestinal injuries or type and location of the IEC along the alimentary tract.

With regard to polymorphism in HSP70gene, a single nucleotide polymorphism in heat shock protein 70-2 (HSP70-2) has been shown to be associated with a severe clinical course in Crohn's disease (CD), but it is not

known if such a relationship exists in ulcerative colitis (UC). Expression of HSP70 was enhanced in ulcerative colitis ($P<0.05$), less so in Crohn's disease and infectious colitis. Mucosal and submucosal mononuclear cells showed enhanced HSP70c expression in Crohn's disease and to a lesser degree in ulcerative colitis while strong epithelial staining of HSP70c and HSP70i in both diseases was found.[13]

Lu XP *et al*[14] showed strong immunoreactivity for the iSP70 in the viable and regenerating cells of both the crypt and the villous surface epithelium of the small and large intestine, within and adjacent to the ischaemic necrotic lesions in most cases of ischaemic bowel disease. HSP70 immunoreactivity was expressed explicitly in the viable, 'recovering' cells following ischaemic injury; these cells are apparently in need of 'housekeeping' and active protein and DNA synthesis, thus requiring HSP assistance in protein assembly, folding, and transport.

Sustained expression of cytoprotective intestinal epithelial heat shock proteins (HSPs), particularly HSP27, depends on stimuli derived from bacterial flora. Inflammation caused by infection with various pathogens has been closely linked to HSP synthesis not only in pathogenic microorganisms but also in inflammatory cells such as neutophils, eosinophils and monocytes/macrophages. Macrophages are exposed to a variety of anti-microbial toxic agents including cytokines, lipid mediators, oxygen free radicals and nitric oxide after phagocytosing bacteria and they increase their endogenous HSP synthesis for protection. HSP70 is known to protect cells against TNF-α mediated cytotoxicity. Studies have demonstrated that heat shock proteins (HSP) can block the production of proinflammatory cytokine through inhibition of NF-kappaB and mitogen-activated protein kinase pathways or activate anti-inflammatory cytokines and therefore control the magnitude of the immune response. HSP27 has been considered a powerful inductor of IL-10, a major inhibitor of Th1 response.

It is increasingly evident that intestinal flora plays a role in the pathogenesis of inflammatory bowel diseases (IBDs). Support for microbial involvement in IBD comes not only from animal models of ulcerative colitis (UC) and Crohn's disease (CD) but also from genetic studies of humans showing disease susceptibility genes, such as NOD2/CARD15 and Toll-like receptors, which are thought to be involved in recognition of bacterial products.[15, 16, 17] It has been suggested that inflammation may not be due to a specific pathogen but rather created by dysbiosis, a shift in the balance of healthy flora in favor of proinflammatory microbial species, which can lead to intestinal inflammation.[18]

Cytokines involved in heat shock induction imply an interrelationship among these mediators. Because of their protective role, HSPs can be expected to down-regulate inflammatory cytokines to overcome inflammation. This suggests a mechanism of modulating cytokine production by both NF-kB and MAPK pathways. Several studies have shown that the heat shock response does, in fact, inhibit some cytokine production mediated by NF-kB and modulates MAPK-dependent cytokine production in a specific manner.

Accumulating evidence reveals that HSPs suppress inflammatory gene expression and thereby inhibit the synthesis of inflammatory cytokines to curb inflammation. Blockade of NF-kB or MAPK-mediated inflammatory responses by HSPs or other agents can be of therapeutic significance. However, the actual mechanisms by which HSPs may act to suppress inflammatory cytokine production through these pathways are incompletely understood.[19] More basic research needs to be done to elucidate the actual mechanism pertaining to the IBD pathogenesis, leaving the hope that heat shock proteins could be the target of therapeutic approaches.

REFERENCES

1. Ang D, Liberek K, Skowyra D, Zyclicz M, Georgopoulos C. Biological role and regulation of the universally conserve d heat shock proteins. J Biol Chem 1991; 266:24233-24236.
2. Georgopoulos C,Welch WJ. Role of major heat shock proteins as molecular chaperones. Annu Rev Cell Biol 1993; 9:601- 643
3. Wu T, Tanguay RM. Antibodies against heat shock proteins in environmental stresses and diseases: friend or foe? Cell Stress Chaperones. 2006;11:1-12
4. Santoro MG. Heat shock factors and the control of the stress response. Biochem Pharmacol. 2000; 1:59:55-63
5. Han SI, Oh SY, Woo SH, Kim KH, Kim JH, Kim HD, Kang HS. Implication of a small GTPase Rac1 in the activation of c-Jun N-terminal kinase and heat shock factor in response to heat shock. J Biol Chem. 2001; 276:1889-1895
6. Kaufmann SHE: He at shock proteins and the immune response. Immunol Today 1990; 11:129-136
7. Hu S, Ciancio MJ, Lahav M, Fujiya M, Lichtenstein L, Anant S, Musch MW, Chang EB. Translational inhibition of colonic epithelial heat shock proteins by interferon-gamma and TNF-alpha in intestinal inflammation. Gastroenterology. 2007; 133: 1893–1904
8. Diether L, Stahl M, Ibrahim ET, Wenzel BE, Drabicki D, Wecke A, Fellermann K, Stange EF. Enhanced intestinal expression of heat shock protein 70 in patients with inflammatory bowel diseases. Digestive Diseases & Sciences 1999; 44:1440–1447
9. Barton S.G.R.G., Rampton D.S, Winrow V.R., Domizio P.,

and Feakins R.M. Expression of heat shock protein 32 (hemoxygenase-1) in the normal and inflamed human stomach and colon: an immunohistochemical study. Cell Stress & Chaperones 2003; 8:329–334

10. Oates PS, West AR.Heme in intestinal epithelial cell turnover, differentiation, detoxification, inflammation, carcinogenesis absorption and motility. World J Gastroenterol. 2006;12:4281-4295
11. Jin M, Otaka M, Otani S, Okuyama A, Itoh S, Iwabuchi A, Sasahara H, Fujimori S, Itoh H, Tashima Y, Masamune O. Effect of pre-induction of heat shock proteins on indomethacin-induced small-intestinal lesion in rats. J Gastroenterol. 1997;32:34-39
12. Klausz G, Molnár T, Nagy F, Gyulai Z, Boda K, Lonovics J, Mándi Y. Polymorphism of the heat-shock protein gene Hsp70-2, but not polymorphisms of the IL-10 and CD14 genes, is associated with the outcome of Crohn's disease. Scand J Gastroenterol. 2005;40:1197-204
13. Lu XP, Omar RA, Chang WW. Immunocytochemical expression of the 70 kD heat shock protein in ischaemic bowel disease. J Pathol. 1996; 179:409-413
14. Sartor R.B. Intestinal microflora in human and experimental inflammatory bowel disease. Curr. Opin. Gastroenterol. 2001; 17: 324–330
15. Hugot JP. CARD15/NOD2 mutations in Crohn's disease. Ann N Y Acad Sci. 2006; 1072:9-18
16. Gazouli M, Zacharatos P, Mantzaris GJ, Barbatis C, Ikonomopoulos I, Archimandritis AJ, Lukas JC, Papalambros E, Gorgoulis V. Association of NOD2/CARD15 variants with Crohn's disease in a Greek population. Eur J Gastroenterol Hepatol 2004; 16P: 1177-82
17. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. Gut 2004; 53:1–4.
18. Malago JJ, Koninkx JF, van Dijk JE. The heat shock response and cytoprotection of the intestinal epithelium. Cell Stress Chaperones. 2002; 7:191-199